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Letter to the Editors

What is a relevant statin concentration in cell experiments claiming pleiotropic effects?

Linda Björkhem-Bergman,[1] Jonatan D. Lindh[1] & Peter Bergman[2,3]

[1]Department of Laboratory Medicine, Division of Clinical Pharmacology, [2]Department of Laboratory Medicine, Division of Clinical Microbiology and [3]Department of Medicine, Center for Infectious Medicine (CIM), Karolinska University Hospital, Huddinge, Karolinska Institutet, Stockholm, Sweden

Apart from their lipid-lowering effects statins have been ascribed a number of other functions including anti-inflammatory and immunomodulatory properties [1]. Notably, most available evidence for the proposed pleiotropic effects of statins is based on *in vitro* studies. We claim that the results from these *in vitro* studies are potentially misleading, because of the high statin concentrations used.

We performed a systematic literature search identifying *in vitro* experiments where mechanistic insights behind pleiotropic effects of statins are claimed. Statin concentrations in these cell experiments were compared with concentrations detected in human plasma. We focused on studies published in journals with an impact factor >15, as these reports were likely to have a broad impact on this field of research. Indeed, the choice of statin concentration is frequently based on previous experiments from papers in high-impact journals. In these articles, pleiotropic effects of statins were only detected at statin concentrations of 1–50 μmol L^{-1} (e.g. [2–6]). In contrast, the mean concentration of statins in human serum (at therapeutic doses) is only 1–15 nmol L^{-1} (Table 1). In addition, the protein binding of statins in human blood is high, 95–99%, and it is only the free fraction (0.01–0.5 nmol L^{-1}) that is pharmacologically active.

Compared with the mean statin concentration in human blood, the peak concentration (C_{max}) is 2–6 times higher (Table 1), but this concentration is only present in serum for a very short time (minutes) while the *in vitro* incubations have lasted for up to 4 days (Table 1).

The large discrepancy between statin concentrations employed in cell experiments and those detected in human plasma is remarkable and, in our opinion, unjustified. The potential problems associated with using too high concentrations *in vitro* can be illustrated by data from our own laboratory where we have found that simvastatin inhibits growth of pneumococci with a MIC value of 15 μg ml^{-1} (36 μmol L^{-1}; unpublished data). This MIC value is approximately in the same range as for other antibiotics (tetracycline MIC = 1 μg ml^{-1}, vancomycin MIC = 2 μg ml^{-1}). Hence, one could make the assumption that statins may be used as antibiotic drugs. However, when the true statin concentration *in vivo* (>1000-fold lower) is considered, statins no longer appear attractive as anti-infective agents.

A possible rationale for using 1000-fold higher concentrations of statins *in vitro* than *in vivo* could be if the drug accumulated in the target organ. Indeed, one study demonstrated statin concentrations in liver tissue exceeding those in serum, but the difference was only twofold [7]. In contrast, the concentration in brain and muscle tissue is only one-third of that in serum [7, 8]. The intracellular concentration of statins in endothelial, blood and immunologically active cells is unknown.

What statin doses have been used in animal experiments? In humans the dosage of statins varies between approximately 0.1–1 mg kg^{-1} bodyweight, while most studies in rodents have used doses of 1–100 or even 500 mg kg^{-1} bodyweight (e.g. [3, 7, 8]). These doses result in concentrations much higher than those achievable in patients [7], better resembling the exposure used *in vitro*. The use of very high doses in rodents is probably possible because of a pharmacodynamic resistance to the pharmacological effect in these animals, e.g. statins do not lower cholesterol concentrations in serum of rodents even though they significantly inhibit the mevalonate pathway in the liver [9].

We fully acknowledge that more clinically relevant doses of statins are used in some animal experiments. However, given that the metabolism and actions of statins differ between humans and rodents, animal experiments claiming that statins are anti-inflammatory should be interpreted with caution.

In conclusion, we believe that a pleiotropic effect of statins is an interesting area that deserves studies on underlying mechanisms both *in vivo* and *in vitro*. However, we do call for an active discussion about relevant statin concentrations in these experiments. If concentrations much higher than those achieved in therapeutic use are chosen, the rationale for this approach should be presented by the authors.

Table 1

Pharmacokinetic and experimental data

Statin	Dose	C_{max} of statin in human serum	Mean concentration in human serum*	Protein binding	Statin concentration in cell experiments† (time of incubation)
Simvastatin: **Simvastatin lactone (inactive prodrug)** **MW: 41 857 g mol^{-1}** **Simvastatin-OH (active metabolite)** **MW: 46 358 g mol^{-1}**	40 mg	19–31 nmol L^{-1} 6–7 nmol L^{-1}	2.2–4.3 nmol L^{-1} [10] 1.6–1.9 nmol L^{-1} [10]	>95%	10 μmol L^{-1} (ND)[2] 1 μmol L^{-1} (30 min) [4]
Atorvastatin **MW: 55 864 g mol^{-1}**	5 mg 20 mg	8 nmol L^{-1} 40 nmol L^{-1}	4 nmol L^{-1} [11] 15 nmol L^{-1} [11]	>98%	10 μmol L^{-1} (48 h) [6]
Lovastatin **(inactive prodrug)** **MW: 40 455 g mol^{-1}**	80 mg	50 nmol L^{-1}	9.4 nmol L^{-1} [12]	>95%	1 μmol L^{-1} (4 days) [5]

*Mean concentration is calculated from AUC values divided by 24 h. †The lowest concentration of statins in cell experiments (published in high-impact journals) associated with a significant pleiotropic effect. MW, molecular weight.

Competing Interests

There are no competing interests to declare.

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CORRESPONDENCE
Dr Linda Björkhem-Bergman, Division of Clinical Pharmacology C1-68, Karolinska University Hospital, Huddinge, Karolinska Institutet, 141 86 Stockholm, Sweden.
E-mail: linda.bjorkhem-bergman@karolinska.se